III. "On a Definite Method of Qualitative Analysis of Animal and Vegetable Colouring-matters by means of the Spectrum Microscope." By H. C. Sorby, F.R.S., &c. Received April 10, 1867.

CONTENTS.

1. *History.*

My attention was first directed to this subject by reading a report of Professor Stokes's very excellent lecture at the Royal Institution, Friday, March 4th, 1864. It immediately occurred to me that a spectroscope might be combined with a microscope, and employed to distinguish coloured minerals in thin sections of rocks and meteorites. I was soon led to examine many other coloured substances, and found that the instrument is more useful in connexion with qualitative analysis, when only very small quantities of material can be obtained. At first I employed the imperfect apparatus described in my Paper in the 'Quarterly Journal of Science' *, but afterwards, along with Mr. Browning, I constructed that described in my Paper in the 'Popular Science Review' †. For general purposes I do not think this could be much improved; but for chemical testing it is much less fatiguing to use a binocular instrument. There were many difficulties to contend with, but at length I constructed one which appears to answer all the requirements of the case.

2. *Apparatus.*

I have an ordinary large binocular microscope, and use an object-glass of about three inches focal length, corrected for looking through glass an inch thick; the lenses being at the top, so as to be as far as possible from the slit. This is placed at the focus, and between it and the lenses, at a distance of about half an inch from them, is a compound prism, composed of a rectangular prism of flint-glass, and two of crown-glass of about 61°, one at each end. This arrangement gives direct vision and a spectrum of the size most suitable for these inquiries, since a wide dispersion often makes the absorption-bands far too indistinct. In order to be able to compare two spectra side by side, a small rectangular prism is fixed over

* April 1865, vol. ii. p. 198. † Vol. v. p. 66.

half the slit, and with the acute angle parallel to and just passing beyond it. This gives an admirable result, the only defect being that, when the spectra are in focus, their line of junction is some distance within it; and therefore to correct this I use a cylindrical lens of about two feet focal length, with its axis in the line of the slit, which can easily be fixed at such a distance between the slit and the prisms, as to bring the spectra and their line of contact to the same focus. In front of the slit, close to the small rectangular prism, is a stop with a circular opening, to shut out lateral light, and a small achromatic lens of about half an inch focal length, which gives a better field, and counteracts the effect of the concave surface of the liquid in the tubes used in the experiments, if they are not quite full. These are cut from barometer-tubes, having an internal diameter of about one-seventh of an inch, and an external diameter of about three-sevenths of an inch. They are made half an inch long, ground flat at each end, and fixed with Canada balsam on slips of glass two inches long and about six-tenths of an inch wide, so that the centre of the tube is about one-fourth of an inch from one edge. By this arrangement the liquid may be examined through the length of the tube by laying the slip of glass flat on the stage of the microscope, or through the side of the tube, by placing the slip vertical and the tube horizontal. Cells of this size can be turned upside down and deposits removed without any liquid being lost; and the upper surface of the liquid is sufficiently flat, even when inclined at a considerable angle. If requisite, small bits of thin glass can be laid on the top, which are held on by capillary attraction, or may be fastened with gold-size, if it be desirable to keep the solution for a longer time. When the depth of colour is too great in the line of the length of the cell, we can at once see what would be the effect of about one-fourth of the colour by turning it sideways; and thus we can save much time, and quickly ascertain what strength of solution would give the best result. Very frequently we obtain an excellent spectrum in one direction with one reagent, and in the other with another, without further trouble. I have constructed a small stage, too complicated to describe in writing, which enables me at once to examine solutions in two such tubes, either endways or sideways, and compare their spectra side by side, or to use test-tubes, or to fix the small apparatus which I have contrived for accurately measuring the spectra. This is of such great importance in these inquiries that I must describe it in some detail.

3. *Scale of Measurement.*

It consists of two small Nicol's prisms, and an intermediate plate of quartz. If white light, passing through two such prisms, without the plate of quartz, be examined with the spectrum-microscope, it of course gives an ordinary continuous spectrum; but if we place between the prisms a thick plate of quartz or selenite, with its axis at 45° to the plane of polarization, though no difference can be seen in the light with the naked eye, the spectrum is entirely changed. The light is still white, but it is

made up of alternate black and coloured bands, evenly distributed over the whole spectrum. The number of these depends on the thickness of the depolarizing plate, so that we may have, if we please, almost innumerable fine black lines, or fewer, broader bands, black in the centre and shaded off at each side. These facts are of course easily explained by the interference of waves. It would, I think, be impossible to have a more convenient or suitable scale for measuring the spectra of coloured solids and liquids. If we use a micrometer in the eyepiece, an alteration in the width of the slit modifies the readings, and the least movement of the apparatus may lead to error, whereas this scale is not open to either objection. Besides this, the unequal dispersion of the spectrum makes the blue end too broad, so that a given width, as measured with a micrometer in the eyepiece, is not of the same optical value as the same width in the red. The divisions in the interference-spectrum bear, on the contrary, the same relation to the length of the waves of light in all parts of the spectrum, and no want of adjustment in the instrument alters their position. As will be seen from the drawing (fig. 1), the unequal dispersion makes the distance betweeen the bands in the blue about twice as great as in the red. The perfection of a spectrum would be one in which they were all at equal intervals; but possibly no such uniform dispersion could be produced. By having a direct-vision prism, composed of one of flint-glass of 60°, and two of crown-glass of suitable angle, we can place it over the eyepiece, and may diminish the dispersion at the blue end, or increase that at the red end, by turning it in one position or the other, and thus see either end to the greatest advantage. It is, of course, very easy to draw spectra on this principle, and give all parts equal prominence, and not an unduly compressed red, and an unduly expanded blue end. Thus drawn, the spectra are far more uniform in many of their characters, and some general laws are at once apparent that otherwise might have been entirely overlooked; and on this account I shall adopt this system in those figured in this paper. It is, in fact, merely representing the actual measurements by drawings, without being at the trouble of distorting them, so as to be like naturally distorted spectra.

Since the number of divisions depends on the thickness of the interference-plate, it became necessary to decide what number should be adopted. At first I thought that ten would be most suitable; but, on trying, it appeared to me too few for practical work. Twenty is too many, since it then becomes extremely difficult to count them. Twelve is as many as can be easily counted; it is a number easily remembered, gives sufficient accuracy, and has a variety of other advantages. With twelve divisions the sodium-line D comes very accurately at $3\frac{1}{2}$; and thus, by adjusting the plate so that a bright sodium-line is hid in the centre of the band, when the Nicol's prisms are crossed, it is accurately at $3\frac{1}{2}$, when they are arranged parallel, so as to give a wider field. The general character of the scale will be best understood from the following figure, in which I have

numbered the bands, and given below the principal Fraunhofer lines.

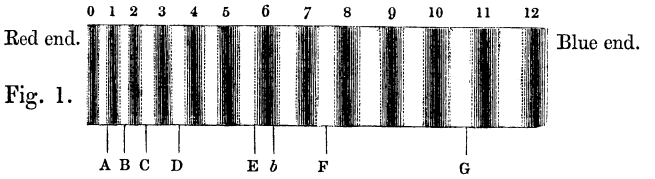


Fig. 1.

The centre of the bands is black, and they are shaded off gradually at each side, so that the shaded part is about equal to the intermediate bright spaces. Taking, then, the centres of the black bands as 1, 2, 3, &c., the centres of the bright spaces are $1\frac{1}{2}$, $2\frac{1}{2}$, $3\frac{1}{2}$, &c., the lower edges of each $\frac{3}{4}$, $1\frac{3}{4}$, &c., and the upper $1\frac{1}{4}$, $2\frac{1}{4}$, &c. We can easily divide these quarters into eighths by the eye; and this is as near as is required in the subject before us, and corresponds as nearly as possible to $\frac{1}{100}$ part of the whole spectrum, visible under ordinary circumstances by gaslight and daylight. Absorption-bands at the red end are best seen by lamplight, and those at the blue end by daylight.

On this scale the position of some of the principal lines of the solar spectrum is about as follows:—

A .... $\frac{3}{4}$	B .... $1\frac{1}{2}$	C .... $2\frac{3}{8}$	D .... $3\frac{1}{2}$
E .... $5\frac{11}{16}$	*b* .... $6\frac{3}{16}$	F .... $7\frac{1}{2}$	G .... $10\frac{5}{8}$

At first I used plates of selenite, which are easily prepared, because they can be split to nearly the requisite thickness with parallel faces; but I found that its depolarizing power varies so much with the temperature, that even the ordinary atmospheric changes alter the position of the bands. Quartz cut parallel to the principal axis of the crystal is so slightly affected in this manner, as not to be open to this objection, but is prepared with far greater difficulty. The sides should be perfectly parallel, and the thickness about ·043 inch, and gradually polished down with rouge until the sodium-line is seen in its proper place. This must be done carefully, since a difference of $\frac{1}{10000}$ inch in thickness would make it decidedly incorrect. I have prepared such plates, corresponding to my own, and placed them in the hands of Mr. Browning and of Messrs. Becks, so that any one wishing to adopt a similar scale may be able to do so more accurately.

The two Nicol's prisms and the intervening plate are mounted in a tube and attached to a piece of brass in such a manner that the centre of the aperture exactly corresponds to the centre of any of the cells used in the experiments, which are all made to correspond in such a manner that any of them, or this apparatus, may be placed on the stage and be in the proper place without further adjustment, which, of course, saves much time and trouble.

4. *Symbols used to describe Spectra.*

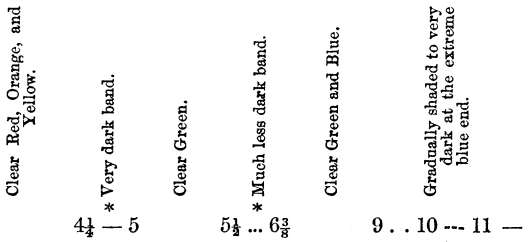
In order to describe spectra in my note-book or in print, I have devised a simple notation, employing types in constant use. Instead of writing an

account of this system, I here give a printed illustration, which will show that by this means it is easy to give in a single line all the essential particulars which would otherwise require a long and tedious description, or a number of drawings and woodcuts. Without some such method of measuring and recording spectra it would be almost impossible to carry on extensive inquiries.

The intensity of the absorption is expressed by the following types:—

Not at all shaded		Blank space.
Very slightly shaded	. . .	Dots with wide spaces.
Decidedly shaded	. . .	Dots closer together.
More shaded	...	Very close dots.
Strongly shaded, but so that a trace of colour is still seen	---	Three hyphens close.
Still darker	—	Single dash.
Nearly black	——	Double dash.

Except when specially requisite, only the symbols . . . --- — are employed for the sake of simplicity, and then as signs of the relative, rather than of the absolute, amount of absorption; and it is assumed that there is a gradual shading off from one tint to the other, unless the contrary is expressed. This is done by means of a small vertical line over the figure (see No. 11), which shows that there is a well-marked division between them. Definite narrow absorption-bands are indicated by * printed over their centre. This will be better understood by a description of the spectrum of deoxidized hæmatin.



The following examples will show how simple or more complicated spectra may thus readily be printed and compared. I have chosen solutions of similar tint, in order to show that the spectra of those of nearly the same colour may be very different, or, if analogous, may differ in details easily expressed by the symbols. The colour of each is given after the name. Nos. 1, 8, 9, 10, 11, 12, and 13 can be kept for a long time sealed up in tubes, and the rest are easily prepared. I have in all cases chosen that strength of solution which gives either the most characteristic spectra, or those best suited for comparison with other allied colours.

1. Cudbear in alum (*Pink*) 3 . . . . . 8 11 . —
2. Colour of Elder berries with citric acid. (*Red Pink*) 4 . - 5¼—8 - - 9 . . . 11 - —
3. Brazil-wood, with bicarbonate of ammonia. (*Pink*) $4\frac{1}{2} - 5\frac{3}{4}$ 8
4. Logwood, with bicarbonate of ammonia. (*Pink*) $3\frac{5}{8} - 5\frac{1}{4}$ 7

The next four are spectra of blood, produced by the successive addition of the various reagents, as in detecting fresh stains.

5. Fresh Blood. (*Pale Scarlet*) $3\frac{1}{2}$ — $4\frac{3}{8}$ $4\frac{3}{4}$ — $5\frac{3}{4}$ 7 . . 8 - - 9—

6. Citric Acid then added. (*Pale Brown*) $1\frac{5}{8}$ . . . $2\frac{1}{4}$ 4 . . . 8 . . . 9 --- 10 —

7. Ammonia then added. (*Pale Brown*) $3\frac{5}{8}$ . . . $4\frac{3}{8}$ $4\frac{7}{8}$ . . . $5\frac{5}{8}$ 7 . . 8 --- 10—

8. Deoxidized hæmatin, from blood-stain 2 years old. (*Pink*) $4\frac{1}{4}$ — 5 $5\frac{1}{2}$ . . . $6\frac{3}{8}$ 9 . . 10 --- 11 —

With these may be compared the two spectra which more nearly resemble those produced by blood than any I have yet seen.

9. Cochineal in alum. (*Pink*) $3\frac{3}{8}$ — $4\frac{1}{2}$ . . . $5\frac{1}{8}$ --- $6\frac{1}{8}$ . . . $7\frac{1}{2}$

10. Alkanet-root in alum. (*Pink*) $3\frac{1}{2}$ — $4\frac{3}{8}$ $5\frac{1}{4}$ . . . $5\frac{3}{4}$

The following spectra of compounds derived from chlorophyll are as complicated as any I have met with.

11. Normal chlorophyll in alcohol. (*Deep Green*) $\frac{7}{8}$ — $2\frac{3}{8}$ --- $3\frac{1}{4}$....$4\frac{1}{2}$ $6\frac{3}{4}$ . . - $7\frac{1}{2}$—

12. Ditto, as decomposed by acids, or as found in some leaves. (*Olive Green*) 1—$2\frac{1}{8}$ $2\frac{3}{4}$ --- $3\frac{3}{8}$ $4\frac{1}{4}$ . . . $5\frac{1}{4}$ --- $5\frac{3}{4}$ . . . $6\frac{3}{8}$ --- $7\frac{3}{8}$ $8\frac{1}{2}$ . . - $9\frac{1}{2}$—

13. Ditto, as decomposed by caustic potash, and then by hydrochloric acid. (*Red-Green, Neutral Tint*) $\frac{1}{4}$ — $\frac{3}{4}$ $1\frac{1}{8}$ — $1\frac{3}{8}$ $1\frac{7}{8}$ — $2\frac{1}{8}$ $4\frac{1}{2}$ — $5\frac{1}{4}$ . . . 9 - - 10—

5. *General Remarks on Absorption, &c.*

It appears to me that in adopting the undulatory theory of light it greatly simplifies the subject before us if we, to some extent, make use of the phraseology of acoustics. And thus, for example, I shall speak of two absorption-bands that occur, one nearer the *red*, and the other nearer the *blue*, end of the spectrum, as being relatively *lower* and *higher*. In a similar manner, if the addition of some reagent cause the absorption to increase towards the blue, and decrease towards the red, end, I shall describe it as *raising* the position of the absorption. We may also make some facts more intelligible by comparing them with the analogous phenomena of sound, and thus, for instance, may suppose that very narrow absorption-bands indicate that the ultimate particles of the substance will only take up vibrations of light of nearly one particular velocity, and that broad absorption-bands show that the particles have a much less definite rate of movement. Analogy would also lead us to infer that, when two spectra differ very decidedly, they must be due to different substances, or to the

same in a different condition; whereas, if two spectra agree, they may be the same substance, or two distinct substances, whose different actions are made equal by particular circumstances. As an illustration, we may refer to a short string, which may give the same note as a longer whose tension is greater. For this reason we should be careful not to rely too much on one spectrum. If, however, we can produce some great physical change in both substances, and still their spectra remain the same under equal conditions, and if this occur uniformly in several different changes, we may conclude that they are identical. Hence the value of the various reagents named below. Many excellent illustrations of these principles could easily be given.

6. *General Method of Experiments.*

Since the spectrum-microscope enables us to use very small quantities, it appeared desirable to adopt such a method of research as would enable us to take full advantage of this circumstance, and to avoid as much as possible previous chemical manipulations. On this account I shall say nothing about modified chemical methods, which may, of course, be also employed when sufficient material is at command. My aim has been to contrive a special system of qualitative analysis of coloured substances applicable to minute quantities, and as independent of general chemistry as the blowpipe method is in the case of minerals. I may here say that in some very important practical applications to the detection of blood-stains not above $\frac{1}{100}$ of a grain was at disposal, and yet perfectly satisfactory results were obtained.

I was led to study the colouring-matters of flowers, leaves, fruits, woods, and roots, because it appeared a most admirable field of inquiry to teach the general principles of the subject. The colours being so various, and occurring under such complicated conditions, I thought that if methods could be devised to distinguish those that are dissimilar and to prove the identity of those that are alike, even when mixed with coloured impurities, such principles could easily be applied to other inquiries. If the question were merely to distinguish or compare absolutely pure colouring-matters, there would be little or no difficulty; but it appeared to me that one great value of the method would be to be able to apply it at once to very impure and mixed materials. In such cases mere colour is of very secondary importance, since that may be totally changed by a very small amount of impurity.

7. *Preparation of Colours.*

If the petals, leaves, &c. of plants be crushed in water, it very commonly happens that the colour is rapidly decomposed and no clear solution can be obtained; but if crushed in a moderate quantity of spirits of wine, and the solution squeezed out, filtered, and evaporated to dryness at a gentle heat, the colouring-matter does not decompose, even

when redissolved in water and filtered to remove anything not soluble in that liquid. This clear solution should then be evaporated to dryness at a gentle heat in a small saucer, and kept *dry*; for then the colours often undergo no important change in the course of many months, whereas, when kept dissolved in water or alcohol, they may quickly decompose. I have thus prepared the colouring-matter of above a hundred different vegetable substances, some of which have become entirely changed, but a large number are apparently still unaltered. I have also kept a number of colours, sealed up in glass tubes, ready for direct examination, dissolved in alcohol, in strong syrup, or in alum. Many have decomposed, but many have kept perfectly well, or have merely faded, and still give excellent spectra after above a year. I have also prepared and kept in the same manner some animal colouring-matters, but comparatively few.

8. *Method of examination.*

The coloured substances are examined, when dissolved in water, alcohol, or other solvent, in the small glass cells already described, and the various reagents are added and mixed by means of a moderately stout platinum wire, flattened at one end and turned up square, like a little hoe. I have made many experiments in order to ascertain what reagents are most serviceable in developing characteristic spectra, and have at length concluded that for general purposes the following are the most convenient. Those which are solid are best kept in small bottles as coarse powder, and added to the small cells in a solid state, so that the quantity used may be more readily known.

9. *Reagents.*

Hydrochloric acid.
Citric acid.
Benzoic acid.
Boracic acid.
Bicarbonate of ammonia.
Carbonate of soda.
Diluted solution of ammonia.
Caustic potash.
Sulphite of soda.
Sulphate of protoxide of iron.
Alum.
Iodine dissolved in alcohol.
Bromine dissolved in water.
Solution of hypochlorite of soda.
Permanganate of potash.

This list might of course be very much extended, if we were to include such reagents as may be used in separating or decomposing colours by the ordinary chemical methods. In describing the effect of those named in

this list, I feel that I could not avoid mentioning some well-known facts without breaking the thread of my argument; and therefore I trust it may not be thought out of place if I give a general account of the whole from the particular point of view required by the subject more especially before us.

The action of many reagents is so intimately related to different parts of the spectrum, as to show that there must be some connexion between so-called chemical reactions and optical phenomena. Not that their effect is absolutely the same in the case of all coloured substances, but generally only the *extent* differs, whilst the *character* of the change is uniform; unless, indeed, decomposition take place; and even then it has a tendency to conform to a general law.

10. *Solvents.*

Water and alcohol are the most useful solvents, and the spectra of the two solutions of the same substance often differ most strikingly; in fact they often behave in other respects as if they were solutions of different substances. Sometimes the spectra are absolutely identical, but often well-marked narrow absorption-bands are seen in the alcoholic solution, where they are almost, or quite, invisible in the aqueous. Very commonly the same bands are seen in both, but not exactly in the same place, alcohol sometimes raising them to a higher part of the spectrum, and sometimes depressing them. Occasionally the spectrum of the dry material is like that of the alcoholic solution, and unlike that of the aqueous, as if the difference were due to the presence of water, but in other cases it is unlike both. At all events the facts clearly show that a solvent has a most important action on the ultimate particles of the substance in solution, since it may produce a greater change in optical phenomena than even chemical combination. Undistilled hard water may act like a weak alkali.

11. *Acids and Alkalies.*

As far as optical phenomena are concerned, there is no absolute division between acids and alkalies; for we have every connecting link from the strongest acids to the strongest alkalies. In order to understand their action, it is most essential to distinguish between what may be called "general absorption" and "local absorption-bands." There may, perhaps, be no absolute line of division, but when seen to advantage they are affected in such a different manner that it is desirable to treat of each separately.

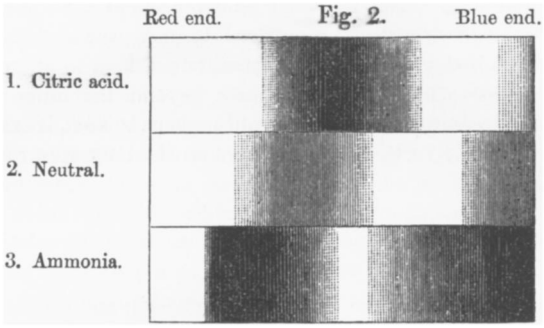
12. *General Absorption.*

As a good example of simple general absorption, we may take the crimson colouring-matter of the common Wallflower (*Cheiranthus Cheiri*), which is soluble in water, and, along with a yellow only soluble in alcohol, gives rise to the varied colours of the flowers.

When neutral, it is crimson....			$2\frac{3}{4}$	....	7		10	..	11	- —
With ammonia, fine green	$1\frac{3}{4}$	—	$4\frac{1}{2}$	-..	6	7	..	8	-	9—
With citric acid, deep pink			$3\frac{1}{2}$	.-	$4\frac{1}{2}$	— 6	-..	$8\frac{1}{2}$		11...

These facts will be better understood by means of the following drawing:—

Fig. 2.



Whence it will be seen that citric acid raises and greatly increases the central absorption, and ammonia lowers and also increases it. At the same time the absorption at the extreme blue end of the spectrum is raised by the acid almost to beyond the range of vision, but lowered to the centre of the spectrum by ammonia. Acids and alkalies of intermediate character, as, for example, boracic acid and bicarbonate of ammonia, produce intermediate effects. These well-known phenomena may be looked upon as typical of acids and alkalies, but the extent of their action varies for each particular colouring-matter, so that in some cases it is slight, and sometimes neither acids nor alkalies produce any effect. Their relative action on the central and upper absorption also varies very greatly in different colours. If there is no general absorption in the centre of the spectrum, when the colour is neutral, but only an absorption at the blue end, acids and alkalies act on it in precisely the same manner as on the absorption at the blue end in the case just described, raising or lowering it to an extent varying greatly according to the substance; and the same may be said of any general absorption at the red end. The reverse certainly occurs when an acid is added to chromate of potash, or excess of ammonia to a salt of copper; and, according to Stokes (Phil. Trans. 1862, p. 609), alkaloid bases usually show this reverse action. It may depend on the different properties of two distinct *compounds*, which does not appear to be the cause of the phenomena now under consideration. In the case of all the vegetable colouring-matters which I have examined, the tendency of acids is to raise, and of alkalies to depress, the general absorption in each part of the spectrum; the extent of this action depending on the strength and quantity of the reagents, and on the nature of each colouring-matter; and thus we have a general rule, and not several, as commonly adopted by chemists, each of very limited application; for instance, that vegetable blues are turned red by acids, and green by alkalies; and that vegetable yellows are reddened by alkalies. I may here remark that some colours

would appear to be exceptions, if we did not remember that waves of light, or waves analogous to them, exist beyond the visible spectrum. Thus, for example, when alkalies are added to the yellow solution of Brazil-wood (*Cæsalpinia crista*), it is changed to pink, the absorption being so much lowered that the blues are transmitted; this clear space corresponding to what was probably a clear space beyond the blues visible under ordinary circumstances, but which would perhaps be seen, if examined in the manner described by Stokes in his paper on the long spectrum of electric light*.

13. *Fading of Solutions.*

One striking peculiarity in the action of acids on the solutions of many vegetable colours is, that, when they are in a particular state of acidity, they fade to nearly or quite colourless, without there being any decomposition. This is especially the case with pink colours dissolved in alcohol. It occurs slightly with blue colours, and little, if at all, with yellows. The aqueous solutions change much more slowly, but more and more rapidly the more they are diluted; and frequently attain a permanent depth of colour, which is dark or pale according as the solution is strong or dilute. Of course I here allude to the effect of the *same total amount of colour*, and not to the different effect of the same *quantity* of a strong or dilute *solution*. The alcoholic solutions obtained direct from the flowers often fade so rapidly, and become so nearly colourless, that any one might easily fancy that all the colour was lost by decomposition; and an evaporating dish containing it, might appear merely filled with brownish alcohol, and yet on evaporation the whole dish might be covered with a fine deep colour. The same change may occur over and over again, the deep-coloured solution first obtained soon fading, and the colour being restored by subsequent evaporation.

When such a colour is dissolved in a little water and added to alcohol in an experiment tube, the colour may at first be very deep, but may fade so rapidly that there is scarcely time to observe the spectrum before it passes into that molecular state which does not absorb any of the rays of light. The colouring-matter of the flowers of the red Salvia (*S. splendens*) is an excellent example. Neutral solutions do not undergo this rapid change; a different condition of acidity is requisite for different colouring-matters; and some do not change at all. A large excess of citric acid very often restores the intensity of the colour; and usually the absorption-bands are seen to the greatest advantage when the solution is in that state which rapidly fades; and by adding too much colour and watching whilst it fades, they may be seen and measured when at their best. This fading of a dark-coloured solution must not be confounded with the change which takes place on diluting some salts, as described by Dr. Gladstone in his paper on that subject†.

* Phil. Trans. 1862, p. 599.

† Quart. Journ. Chem. Soc. vol. xi. p. 36.

14. *Absorption-bands.*

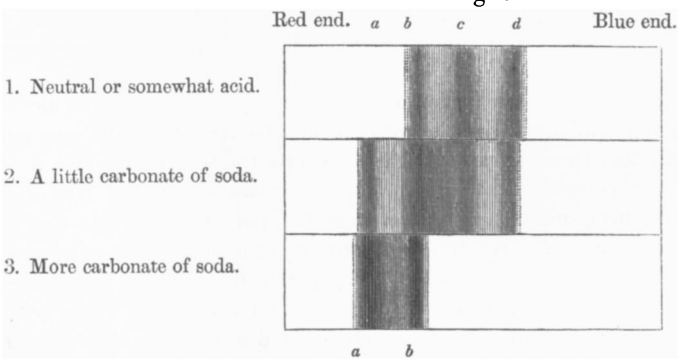
Though acids and alkalies thus, to a greater or less extent, alter the position of the general absorption, they act very differently on the special, local absorption to which it is very convenient to restrict the term "absorption-bands." Since I shall often have to speak of their being at equal intervals, it would be well to say that I have found it convenient to construct a wedge-shaped piece of quartz, cut parallel to the axis of the crystal, and to use it along with two Nicol's prisms in such a manner that the spectrum may be divided into any requisite number of equal portions, by interference-bands situated in any requisite position. This of course avoids the errors which so often happen when we compare together measurements that cannot be made with very great accuracy.

As an excellent illustration I select the colouring-matter of Alkanet-root (*Anchusa tinctoria*). It is insoluble in water, but is easily dissolved by alcohol, even when much diluted with water, and gives a clear pink solution. The spectrum is nearly the same when the colour is dissolved in absolute alcohol, as when much water is present, only each of the absorption-bands is situated rather higher. Thus, taking the centres of the bands, we have—

	b.	*c*.	*d*.
Absolute alcohol	$4\frac{3}{8}$	$5\frac{7}{8}$	$7\frac{3}{8}$
Very dilute alcohol	$4\frac{1}{4}$	$5\frac{3}{4}$	$7\frac{1}{4}$

The general spectrum of the solution in dilute alcohol will be best understood from the following figure, No. 1 :—

Fig. 3.



Acids produce no important change, and the effect of alkalies is best seen by gradually adding carbonate of soda. This alters the colour to a more and more blue-purple, and the spectrum is changed in the manner shown in fig. 3. The three bands seen in the neutral solution may be referred to as *b*, *c*, and *d*, and their centres occur at equal intervals of about $1\frac{1}{2}$. When enough carbonate of soda has been added to make it slightly purple, a fourth band, *a*, makes its appearance, separated from *b* by the same equal interval of $1\frac{1}{2}$, whilst the other bands remain in the same

position as at first, only modified in intensity. The band *a* becomes darker and darker as more carbonate is added, until, when the solution is a fine purple, it is as dark as the others (see No. 2); and on adding more carbonate it becomes still darker, and the bands *c* and *d* more faint, until the solution is a purple-blue; and the spectrum has only the two well-marked bands *a* and *b*, shown by No. 3.

The bright blue colouring-matter of the flowers of *Lobelia speciosa* gives, when *neutral*, almost exactly the same spectrum as that of Alkanet-root when *alkaline*, No. 3, having two well-marked absorption-bands, whose centres are at $2\frac{3}{4}$ and $4\frac{1}{4}$; and on adding carbonate of soda, the upper one is gradually removed, and the centre of the lower is depressed to near $2\frac{1}{2}$. More or less similar results occur in the case of many other blue colouring-matters; and on adding a slight excess of acid the general absorption is raised, and other bands may be developed higher up, at equal intervals; but when a large excess has been added, they are lost in a strong general absorption. Too strong an alkali may also destroy narrow bands in a similar manner, as is well seen in the case of Brazil-wood. The neutral aqueous solution shows an absorption-band, made far more distinct by the addition of bicarbonate of ammonia, which makes it pink and very fluorescent. The spectrum is then

$$4\overset{*}{\frac{3}{4}}—5\frac{3}{4} \ldots\ldots 7;$$

but on adding excess of ammonia the solution ceases to be fluorescent, the narrow absorption-band is lost, and the spectrum becomes

$$3\tfrac{1}{2} \ldots 4\tfrac{1}{4}—8 \ldots 9\tfrac{1}{2}.$$

Ammonia does not produce this effect when the colour is dissolved in alcohol, the solution remaining fluorescent and still giving the narrow band; and, as a general rule, that solvent greatly impedes or entirely prevents such changes, and on this account almost invariably shows absorption-bands to the greatest advantage.

We therefore see from the above examples that the absorption follows the general rule, and is raised by acids, and depressed by alkalies; but this only applies to the absorption when viewed as *a whole*, and not to the separate bands; for those reagents change their *intensity*, but not their *position*. In some cases, indeed, their position is slightly altered, so that perhaps it would be more correct to say that acids and alkalies may raise and depress the general absorption to an extent equal to a considerable fraction of its own great breadth; whereas they either do not change the position of narrow absorption-bands, or merely raise and depress them by a fraction of their own narrow width. It is their very definite position that makes them so useful in this method of analysis.

Unfortunately I have not hitherto been able to find a sufficient number of colouring-matters giving rise to three or more well-marked absorption-bands, to warrant a general conclusion; and therefore it is perhaps premature to conclude that their centres always occur at *equal intervals*. At

the same time it is certainly a very common fact. When the maximum point of transparancy occurs between the different bands, there may be, as it were, a double interval; but then, sometimes, even this missing band may be seen under favourable conditions. A difference in the general absorption may also somewhat alter the apparent position of a band, if it is strongly shaded on one side, and not on the other; and the presence of impurities may also modify the results, so that absolute accuracy cannot be expected in all cases; and occasionally very narrow bands occur which appear to belong to a second system. It must be borne in mind that the bands are equidistant, only when measured by means of the interference-spectrum. Thus, in the case of Alkanet-root, when measured with a micrometer, instead of the intervals *a* to *b* and *c* to *d* being equal, they are related to one another about as $1\frac{1}{2}$ and 2, and thus the general law is entirely obscured. If subsequent research should prove that the bands are normally at equal intervals, it will be a fact of great value in deciding whether certain spectra are or are not due to a *mixture of colours*; since, if a band occurred at a perfectly unequal interval, it would show that there must be at least two substances. Even in the present state of our knowledge, any inequality should make us carefully search for some satisfactory reason for such a divergence from a common rule. My meaning will be better understood from the following examples.

If a little of the colour of Brazil-wood be added to the solution of Alkanet-root, the bands are not altered, and are seen at $4\frac{1}{4}$, $5\frac{3}{4}$, and $7\frac{1}{4}$; but a little bicarbonate of ammonia developes a well-marked band, whose centre is at $5\frac{1}{4}$, and therefore at an interval of 1, instead of $1\frac{1}{2}$. The same is also well seen in the case of a mixed solution of Brazil-wood and blue *Lobelia*. I therefore argue that if an unknown substance gave rise to similar spectra, with bands at unequal intervals, we ought strongly to suspect that it was either naturally composite, or that some new compound had been formed by decomposition. As a very good illustration I may refer to the product of the action of acids on chlorophyll. The band in the red is not at an equal interval; but, on careful examination, it is seen to be made up of two bands, the upper of which is at an equal interval. I was not aware that these were due to two different substances, but was led to think it very probable; and Professor Stokes informs me that he has proved it to be the case. As an illustration of another kind of exception, I refer to the colouring-matter of the pink Stock (*Matthiola annua*). The aqueous solution shows two bands, whose centres are at about $3\frac{5}{8}$ and $5\frac{1}{4}$; and on adding ammonia the upper is removed, and the lower depressed to $3\frac{1}{4}$. In spirit of wine they are at 4 and $5\frac{1}{2}$, and ammonia developes a third at 3, which are *not* equal intervals. However, if absolute alcohol be used, the bands are at $2\frac{3}{4}$, $4\frac{1}{2}$, and $5\frac{1}{2}$, which are *equal* intervals; and thus we see that the abnormal inequality is due to the presence of water, which causes the spectrum to be as if due to a mixture of two colours, when in reality it is the same colour dissolved in two solvents.

In spectra showing one absorption-band, there is very commonly a general absorption, extending from it towards the blue end; whereas it so seldom extends towards the red end that it is doubtful if it ever occurs in substances, undoubtedly not a mixture of two colours. It can, however, so easily occur in mixed colours, that any substance giving rise to such a spectrum is probably a mixture. Many illustrations might be given, but I will select Brazil-wood, and the same artificially mixed with the colour of beet-root. Adding bicarbonate of ammonia to both, we have—

Brazil-wood alone	$4\frac{3}{4}$—$5\frac{3}{4}$ . . . 7
Brazil-wood and beet-root	$3\frac{1}{2}$...$4\frac{5}{8}$—$5\frac{3}{4}$ 8

Here, then, the shading below the absorption-band from $3\frac{1}{2}$ to $4\frac{5}{8}$ is evidence of the second colour, and if such a mixture had occurred naturally its mixed character might easily have been overlooked. I have found many cases similar to this, and had proved that they were mixtures, before I was aware that the spectra indicated it. If these very common facts turn out to be general laws, we might thus detect at once the presence of as many as three different substances, or at all events might learn what further examination was desirable.

15. *Sulphite of Soda.*

Sulphite of soda is a most valuable reagent, and its action very remarkable. It enables us to divide colours into three groups, according as it produces a change in an ammoniacal, or acid solution, or in neither. The action is related in a very simple manner to the spectra. Having added an excess of ammonia, there may be a well-marked broad absorption over more or less of the red, orange, yellow, and upper green; and above this a clear transparent space, limited by a variable amount of absorption, extending downwards from the extreme blue. Fig. 2 will illustrate my meaning. In the case of one group of colours, the addition of sulphite of soda almost immediately removes the detached, broad absorption in the lower part of the spectrum, but leaves that at the blue end quite unchanged, or only slightly modified by the solution being made more alkaline. If, then, as in the case of magenta, there is no absorption at the blue end, sulphite of soda makes the solution quite colourless; whereas if the blues are absorbed, as in the case of the ammoniacal solutions of the colour of red roses, and some species of *Dianthus*, it changes the colour from green to yellow. If the absorption extends continuously down from the extreme blue to the orange, as often happens when ammonia is added to yellow colours, sulphite of soda produces no change. It is only when there is a more or less perfect *division* between the upper and lower absorption, that it has any effect; and then it simply and entirely removes the lower absorption. Some colours are changed immediately, even when a very small quantity of sulphite is added; but others require more and change gradually, though still very soon.

16. *Groups A, B, and C.*

Colours which are thus altered when the solution is ammoniacal, constitute my group A. Frequently, however, sulphite of soda does not remove the detached absorption when excess of ammonia is present, but does so when there is an excess of citric acid. These constitute my group B. As in the other group, any absorption which extends continuously from the extreme blue end is not altered, but the detached absorption in the green is almost immediately removed; and therefore a deep pink or red solution may at once become quite colourless, or only a very pale yellow; and in many cases this residual colour is due to some yellow colouring-matter mixed with the other. I have never seen a colour which was changed by sulphite when alkaline, and not when acid; and thus citric acid never restores the colour when it is added to the changed ammoniacal solution. Excess of ammonia usually restores the faded acid solution to nearly the original colour, and it is therefore not a case of actual decomposition, but merely the result of some remarkable molecular change. A third group of colours consists of those which are not almost immediately changed by sulphite of soda, when either alkaline or acid; and these I call group C. Some of them may fade on keeping several hours, and some do not fade even in several days, but they cannot thus be divided into two definite groups. When thus faded, ammonia does not restore the colour; and therefore it is evidently the effect of decomposition, and not like the mere molecular change met with in group B.

On the whole, the groups A, B, and C are remarkably distinct. There are, indeed, a few cases where the change takes place somewhat slowly; and a few scarlet colours do not show very distinctly the characteristic peculiarities of either B or C; but there are other very strong reasons for believing that some of these are really mixtures of different groups. Even if it should be found that perfectly simple colouring-matters may have, as it were, intermediate characters, such appear to be so rare that practically they may be classed with mixtures, until some reason be found for classing them together as exceptions.

These reactions of sulphite of soda are so much interfered with by the presence of alcohol, that it should never be employed as a solvent, unless the substance is insoluble in water; and then it should be diluted as much as possible, since the ordinary spirit of wine with an equal quantity of water is the extreme strength admissible, and even that very much delays the reaction. The effect of various other reagents is also sometimes very different, according to the nature of the solvent.

The three groups A, B, and C differ in other particulars. It is easy to change A or B into C by various reagents which produce decomposition, but I do not know a case where C can be changed into A or B. Caustic alkalies usually soon decompose colours belonging to group A, when dissolved in water, but act slowly on those of groups B and C. Usually colours of group C are far more permanent than those of groups A and B,

and to it belong most of the vegetable colours used in dyeing, and nearly all yellows.

17. *Other Reagents.*

Boracic Acid.—The chief value of this reagent is that it gives nearly the same spectrum as that of a neutral solution when added after the addition of a slight excess of ammonia. It should therefore be well fused in a platinum crucible and recrystallized, so as to be quite free from any stronger acid.

Sulphate of Iron.—Sulphate of the protoxide of iron is chiefly useful as a deoxidizing agent, in the case of blood and a few analogous substances, taking care to have citric acid present to prevent the precipitation of the oxide by ammonia *.

Alum.—Alum has a remarkable influence on some colours, and it has the property of gradually restoring many after they have passed into the faded modification. Many colours also may be kept for a long time dissolved in a strong solution, sealed up in tubes; and it is occasionally an excellent solvent for substances insoluble in either water or alcohol. The chief objection to it as a reagent is that the spectra are so much influenced by the presence of ammonia, even when neutralized by an acid, that it is almost impossible to compare together different substances under exactly the same conditions.

Iodine and Bromine.—Iodine dissolved in alcohol, and bromine in water, are useful in producing decompositions, which may differ very considerably in colours which are otherwise very similar; as, for example, the yellow colouring-matters of the root of rhubarb and of turmeric. The iodine or bromine should be added in sufficient quantity, and then ammonia used to remove the excess, and thus avoid the effect of their own colour. The solution may then be made acid with citric acid, and should in both cases be compared with another tube to which no iodine or bromine has been added.

Hypochlorite of Soda.—This reagent, with or without the addition of citric acid, is sometimes useful, as for instance in detecting the adulteration of rhubarb with turmeric; but generally its action is too powerful and too uniform.

Permanganate of Potash.—This also usually acts too powerfully on colouring-matters. The excess can easily be removed by sulphite of soda, which makes an alkaline solution pale yellow, but an acid solution quite colourless.

18. *Grouping of Colours.*

Having now considered some of the chief peculiarities of the most useful reagents, I proceed to describe what appears to me to be the most convenient method of dividing colouring-matters into groups and subgroups, so as to enable us to ascertain the nature of any particular substance under

* See Stokes's Paper, Proceed. Roy. Soc. vol. xiii. (1864) p. 355.

examination. The number of distinct coloured compounds met with in different plants is so great, that some such classification is imperative. In the first place, we cannot do better than divide them according as they are soluble in water or alcohol. This may be looked upon as a *chemical* division, and is very useful in practice. Thus—

Soluble in water and not precipitated by alcohol	Division 1.
Soluble in water and precipitated by alcohol	„ 2.
Insoluble in water but soluble in alcohol	„ 3.
Insoluble both in water and alcohol	„ 4.

Of course cases occur which cannot be unhesitatingly classed with any one of these; but they often form good practical divisions, and necessarily modify the methods requisite for further examination.

19. *Method and Order of Experiment.*

If a colour belongs to division 1, a small quantity, sufficient for three or four experiments, should be exposed to the vapour of ammonia in a watch-glass, until there is certainly no longer any *free acid*, and then gently evaporated, so that all excess of ammonia may be lost. If not thus made neutral we might be entirely misled; for some pink colours are blue reddened by an acid. A small quantity should then be dissolved in water in one of the small experiment-tubes and the spectrum observed. If too little colour has been added to give the characteristic spectrum, more should be introduced; but if any part is entirely absorbed, the cell should be turned sideways, in order to see whether or no some narrow absorption-band occurs there; and then it may be desirable to remove some of the solution, and fill up the cell with water. As a general rule, so much colour should be added as to make the darkest part of the spectrum decidedly shaded, but yet not so black as to hide any narrow bands; and if any occur, the solution should be made of such a strength as to show them to the greatest advantage. This can easily be done, after a little practice, and is made much easier by being able to turn the tubes sideways. Having noted the spectrum of the neutral solution, a very small quantity of ammonia should be added, and then a decided excess, the spectra being examined to see if there be any difference; for this is very often the case and very important facts may be overlooked if too great an excess be added at first. The addition of a small bit of sulphite of soda then shows at once whether or no a colour belonging to group A is present; and on adding excess of citric acid we may also determine whether it chiefly belongs to groups B or C. Ammonia should then be added in excess, which may or may not restore it to the same state as before the addition of the acid. To another portion of colour carbonate of soda should be added, and then excess of citric acid, both spectra being carefully observed; and finally sulphite of soda, which definitely shows whether or no there is any other colour than one belonging to group C. Combining the results

of the two sets of experiments, we may decide whether it belongs to groups A, B, or C, or is a mixture of any of them. If the substance is insoluble in water but soluble in alcohol, the same experiments should be made, only we must add the colour dissolved in alcohol to as much water as can be used without making the solution turbid, and must remember how much the presence of alcohol may interfere with the action of some of the reagents.

Another portion of the neutral colour should then be dissolved in as strong alcohol as will give a clear solution, and ammonia, benzoic acid, a little citric acid, and much of it added one after the other; and all the spectra carefully observed, as well as any other facts which may present themselves.

By thus using three separate quantities of colour, and adding reagents one after the other, we may obtain about a dozen spectra, which may differ from one another in important particulars, or in some few cases may be all alike. The experiments are so easily made, that the whole series of twelve spectra may be seen in the space of five minutes; and the total quantity of material need not in some cases be more than $\frac{1}{1000}$ of a grain. The facts thus learned may show that for particular practical purposes some different method could be employed with advantage, and that only one or two simple experiments are needed. For example, suspected blood-stains should be treated in an entirely different manner, as described in my Paper on that subject *; and in examining dark-coloured wines, in order to form some opinion of their age from the relative quantity of the colour belonging to group C, gradually formed by the alteration of the original colouring-matter of the grape belonging to group B, it is only requisite to observe the effect of sulphite of soda after the addition of citric acid. It would, however, extend this Paper beyond the limits I have prescribed to myself, if I were to enter into practical applications, and I shall therefore merely give a description of a convenient method of grouping the various colours.

20. *Subgroups.*

Since the narrow absorption-bands are decidedly the most important means of identification, it appears to me that we cannot do better than adopt subdivisions founded on *their number*. We may thus divide each group A, B, and C into subgroups, in which the neutral aqueous solutions exhibit 0, 1, 2, 3, &c. decided absorption-bands. Sometimes one of them may be so obscure that we may hesitate whether it should be counted or not; but practically this is no very serious objection, if we decide to reckon only distinct bands, and to look on the fainter as important merely in identifying individual colours. If no absorption-band can be seen in the neutral solution, we may take into account those seen when more or less ammonia is added; and if none occur in either case, we may make use of those seen in the alcoholic solution when neutral, and after the addition of ammonia. Whenever in this order of experiments the solution gives

* Quarterly Journal of Science, vol. ii. p. 205.

any decided absorption-band the subgroup is determined; and it is only when none has been produced that the process must be carried further.

The general connexion of the subgroups will be best seen from the following Table:—

$$1,\ A \begin{cases} aq_0 \\ aq_1 \\ aq_2,\ \&c. \end{cases} \quad \begin{cases} am_0 \\ am_1 \\ am_2,\ \&c. \end{cases} \quad \begin{cases} al_0 \\ al_1 \\ al_2,\ \&c. \end{cases} \quad \begin{cases} am_0 \\ am_1 \\ am_2,\ \&c. \end{cases}$$

The same system is applicable to each division, 1, 2, and 3, and to each group A, B, and C. We can easily express the subgroups by using one or more of the signs *aq, am, al, am,* with a figure to indicate the number of bands in the first term that contains any; those before it being given to show the facts more clearly.

Each colour can be indicated by writing after the subgroup the characteristic spectrum, or, for the sake of simplicity, merely the position of the *centres* of the bands, when they are seen as independent as possible of general absorption. If the centres of the bands are in different positions the colours cannot be the same; but if they agree it does not necessarily follow that they are the same. It is probable, but must be further proved by the correspondence of other spectra. As examples I give a few well-marked cases.

Purple Pansy ..... 1, A, $aq_0\ am_1$ (4).
Crimson Rose ..... 1, A, $aq_0\ am_0\ al_0\ am_1$ ($2\frac{1}{2}$).
Blue Lobelia (*L. speciosa*) ..... 1, B, aq_2 ($2\frac{3}{4}$, $4\frac{1}{4}$).
Pink Stock (*Matthiola annua*) ..... 1, B, aq_2 ($3\frac{5}{8}$, $5\frac{1}{4}$).
Several blue species of *Campanula*..... 1, B, aq_4 ($2\frac{3}{8}$, 4, $5\frac{5}{8}$, $7\frac{1}{4}$).
Brazil-wood (*Cæsalpinia crista*) ..... 1, C, aq_1 ($5\frac{1}{4}$).
Logwood (*Hæmatoxylum campechianum*) 1, C, aq_1 ($4\frac{3}{8}$).
Sandalwood (*Pterocarpus santalinus*) ..3, C, aq_2 (6, $7\frac{1}{2}$).
Alkanet-root (*Anchusa tinctoria*) .....3, C, aq_3 ($4\frac{1}{4}$, $5\frac{3}{4}$, $7\frac{1}{4}$).

21. *Individual Colours.*

Having then ascertained to which subgroup any particular colour belongs, it is in the next place requisite to determine what particular substance it is. When it gives rise to well-marked absorption-bands, this may be more or less definitely decided by their exact position and character; since they may of course occur in different situations, or vary much in absolute and relative breadth and in intensity. Thus, choosing closely related spectra, we have, for example,—

1, B, aq_2
Blue *Lobelia speciosa*..... $2\frac{1}{4}$—$3\frac{1}{4}$...$3\frac{3}{4}$—$4\frac{5}{8}$ 11...
Pink *Matthiola annua* ..... $2\frac{3}{4}$---$4\frac{1}{4}$...$4\frac{1}{2}$---$5\frac{1}{2}$...8 10..11—

1, C, aq_1
Logwood (*Hæmatoxylum campechianum*) $3\frac{5}{8}$—$5\frac{1}{4}$...7--8—
Brazil-wood (*Cæsalpinia crista*) ..... $4\frac{1}{2}$—$5\frac{3}{4}$..7--8—

Such spectra are at once seen to differ most decidedly when compared side by side; and that the colouring-matters are entirely different is proved by other facts. If the absorption-bands agree very closely, we ought to compare other spectra before concluding that the substances are the same.

22. *Mixed Colours.*

Of course, if any impurity is present which absorbs that part of the spectrum where the characteristic bands occur, it may be difficult, or even impossible, to determine the nature of the substance; but the rest of the spectrum may be obscured, and the general colour entirely changed, without the least difficulty being thereby produced. Thus, for example, on adding a solution of Saffron (*Crocus sativus*) to that of the blue *Lobelia*, the colour is changed from blue to a curious olive, and the spectrum becomes—

Lobelia and Saffron.....	$2\frac{1}{4}$—$3\frac{1}{4}$...$3\overset{*}{\frac{3}{4}}$—$4\frac{5}{8}$	$6\frac{1}{2}$..7-—
Lobelia	$2\frac{1}{4}$—$3\frac{1}{4}$...$3\overset{*}{\frac{3}{4}}$—$4\frac{5}{8}$	11...
Difference		$6\frac{1}{2}$..7-—

If we did not know it, we might thus infer that they were the same substance, and only differed because one contained a yellow colour; and this conclusion would be borne out by adding to each citric acid and sulphite of soda, which make the *Lobelia* colourless, and leave the residual yellow colour, $6\frac{1}{2}$..7-—, in the case of the mixture. The petals of very many flowers do really contain more or less of such a yellow, which appears to be that developed to a much greater extent in the stamens, &c.; and though this often modifies the general colour and the spectra, its presence may be recognized in a similar manner. Different species of *Dianthus*, various kinds of Roses, and *Digitalis purpurea* are good examples of one general colouring-matter modified in this manner. Its normal character is

$$1,\ \mathrm{A},\ aq_0\ am_0\ al_0\ am_1\ (1\tfrac{3}{4}\text{--}\overset{*}{2}\tfrac{1}{4}...4\tfrac{1}{2}\quad 11\,..).$$

In studying mixed colours, so much depends on their special characters, that it would be difficult to give any other general rules; and particular cases do not form part of the plan of the present paper.

23. *Spectra with no Bands.*

The principal difficulty to be contended with in this method of qualitative analysis, is in the case of the subgroups where no decided absorption-bands can be developed by any of the reagents. They can be easily divided into the groups A, B, and C, but the difficulty is to distinguish the separate colours, if we are not sure that they are pure and simple. Sometimes special facts may be of use; but, as a general rule, we are compelled

to have recourse to the position and character of the general absorption. This requires a good deal of care, since a difference in the state of the solution may make the same colour differ more than two quite distinct colours. After trying a number of experiments, I find that the best spectra for comparison are those obtained by adding first a moderate excess of carbonate of soda, and then a considerable excess of citric acid. Both of these solutions change very slowly, and give well-marked spectra; whereas ammonia often causes decomposition, and weaker alkalies or acids give much more faint spectra, or such as rapidly fade. Closely related colours should be compared together, and made as nearly equal as possible after the addition of the carbonate, and then citric acid added in considerable, and nearly equal, excess. We thus can compare two different spectra; and even if the position of the absorption is the same in both cases, the relative intensity may vary considerably. Very closely allied colours may often be easily distinguished in this manner, and the only great difficulty is when coloured impurities are present. As an example, I give some colours belonging to subgroup 1, B, $aq_0\, am_0\, al_0\, am_0$.

		Carbonate of soda.	Citric acid.
Petals of Wallflower (*Cheiranthus Cheiri*)	$2\frac{1}{4}$---5	8..9--10—	$3\frac{3}{4}$.....7
Dark grapes	$2\frac{1}{4}$---$5\frac{3}{4}$...9	10..11--	$4\frac{1}{4}$.....$8\frac{1}{2}$
Fruit of Elder (*Sambucus nigra*)	$2\frac{1}{2}$---6..9	11...	$4\frac{1}{2}$.....$8\frac{1}{2}$

The first differs entirely from the latter two, but they are so similar that it requires great care to be sure that they differ essentially. If it were quite certain that such colours were pure, it would not be difficult to distinguish them with confidence; but since they may contain coloured impurities, we must occasionally be content with results somewhat doubtful in questions of minute detail, which might not be of the least importance in some practical investigations.

24. *Yellow Colours.*

One of the best general methods of distinguishing yellow colours belonging to subgroup C, $aq_0\, am_0\, al_0\, am_0$, or those with bands which are much alike, is to make them as nearly as possible of the same tint when neutral, and then to add excess of ammonia, which may make them very unequal. For example—

	Neutral.	Ammonia.
Yellow Dahlia (*D. variabilis*)	8..9--10—	3...4--$4\frac{1}{2}$—
Yellow Calceolaria (*C. aurea-floribunda*)	7...9--11—	$6\frac{1}{4}$..$6\frac{3}{4}$--7—
Saffron (*Crocus sativus*)	7...8--11—	7..8--11—

The action of ammonia thus shows that they differ very much, but at the same time the *Calceolaria* might be a mixture of the other two, and this would have to be decided by other facts.

25. *Fading of Group C.*

Sometimes in examining colours of group C, advantage may be taken of the different rate at which their acid solutions decompose and fade, when a considerable quantity of sulphite of soda has been added to an acid solution. The two solutions should be made as nearly equal as possible in all respects, and then the rate of fading may prove that they are very different, or may show that one is a mixture. After fading, the addition of excess of ammonia may show valuable facts. For example, the colour of the root of the red beet (*Beta vulgaris*) is pink, but that of the leaves is red, the spectrum differing from that of the root merely in having the blue end much absorbed. On keeping acid solutions of both to which sulphite of soda has been added, that of the root becomes colourless, and that of the leaves yellow; and thus, considering that acid solutions of colours belonging to group C are very rarely pink, it is almost certain that the colour of the leaves is the same as that of the root, but modified by the yellow colour so common in leaves.

26. *Conclusion.*

Such, then, is a general outline of the method which I have hitherto found the most convenient in studying different colouring-matters, and for determining to what individual species any particular colour may belong. I need hardly say that it is just the sort of qualitative analysis to employ in detecting adulterations in many substances met with in commerce, as well as in inquiries where very small quantities of material are at command. By this method we might be able in a few minutes to form a very satisfactory opinion, or at least one that might meet all practical requirements, and even under unfavourable circumstances we might narrow the inquiry to a surprising extent; and if this can be said even now, surely further research cannot fail to make it most useful in cases where ordinary chemical analysis would be of little or no use.

The Society then adjourned over the Easter recess to Thursday, May 2.

May 2, 1867.

Lieut.-General SABINE, President, in the Chair.

In conformity with the Statutes, the names of the Candidates recommended for election into the Society were read from the Chair, as follows:—

William Baird, M.D.
W. Boyd Dawkins, Esq.
Baldwin Francis Duppa, Esq.
Albert C. L. G. Günther, M.D.
Julius Haast, Esq., Ph.D.
Captain Robert Wolseley Haig, R.A.
Daniel Hanbury, Esq.
John Whitaker Hulke, Esq.
Edward Hull, Esq.
Edward Joseph Lowe, Esq.
James Robert Napier, Esq.
Benjamin Ward Richardson, M.D.
J. S. Burdon Sanderson, M.D.
Henry T. Stainton, Esq.
Charles Tomlinson, Esq.